Anticonvulsant Effect of *Berberis integerrima* L. Root Extracts in Mice

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Abstract

*Berberis integerrima* is a member of Berberidaceae family. Berberine is one of the main constituents of this plant, having neuroprotective effect on central nervous system diseases. In this study, the anticonvulsant activity of methanolic extract, and hydro-methanolic fraction, and chloroform fraction of *B. integerrima* was assessed. The anticonvulsant effect of *B. integerrima* was investigated using both pentylenetetrazole (PTZ) and maximal electroshock (MES)-induced seizure models. The LD50 value of the methanolic extract was 302.676 mg/kg. In the PTZ test, methanolic extract (140 and 200 mg/kg, p < 0.01), hydro-methanolic fraction (200 mg/kg, p < 0.01), and chloroform fraction (200 mg/kg, p < 0.01) increased the onset time of hind limb tonic extensions (HLTEs). The protective effect against mortality (convulsion survivors/animals tested) was 2/8 in methanolic extract, and 3/8 in hydro-methanolic fraction at a dose of 200 mg/kg and in chloroform fraction at a dose of 140 mg/kg. In the MES test, this plant did not display any significant effect in reducing HLTE duration. According to phytochemical screening, methanolic extract contained alkaloids and tannins. The present study, conducted in mice, indicated that *B. integerrima* has anticonvulsant activity in PTZ-induced seizures. It is concluded that *B. integerrima* may be useful in petit mal epilepsy.

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1. Introduction

Barberry is an evergreen and self-fertile plant that belongs to Berberidaceae family [1,2]. It is a shrub with yellow-to-brown bark, red-colored fruits, and thick and woody roots covered with a brittle bark [3,4]. This plant propagates through the suckers of root [1]. A variety of Berberis sp. are available in Iran, including Berberis vulgaris L., Berberis orthobotrys Bienert, Berberis crataegina D.C., B integerrima, and Berberis khorasanica Browicz [1,5]. The fruits of barberry are used as food flavor [1]. Many of Berberis L. species are used to alleviate insomnia [6], liver disorder, bronchial diseases, and urinary and gastrointestinal discomforts [7], and as an antirheumatic [8], antipyretic, [9], antibacterial [4], and antifungal [7] agent in traditional medicine. Recent pharmacological investigations have shown some pharmacological activities [10], including antimicrobial [11], anti-inflammatory, antinoceptive [12], antihistaminic, anticholinergic [13], potent vasodilatory, and antiarhythmic activities, as well as increasing the duration of action potential in purkinje fibers and ventricular muscles [14] and decreasing morphine dependence, locomotor activity, and inducing hypnosis [15]. Compounds such as berberine chloride, palmatine chloride, oxyacanthine, berbamamine, quaternary protoberberines, and bisbenzylisoquinoline alkaloids are the main constituents of this plant, and berberine alkaloid is mostly found in the roots [2–4,7,8]. Berberine has many pharmacological activities, including its hypotensive, immunostimulating, and sedative properties; it exerts some beneficial effects on central nervous system activities, such as protective effect in Alzheimer’s, cerebral ischemia, mental depression, schizophrenia, anxiety, and depression, by increasing the content of norepinephrine, serotonin, or dopamine in the brain [16–18].

Epilepsy is a neurological illness that is characterized by recurrent seizures, and up to 5% of people develop epilepsy in their lifetime [19]. Although several anticonvulsant drugs are used to treat seizure attacks, about 30% of patients are medicated incompletely. Furthermore, current antiepileptic drugs have toxicity and teratogenic effects, which necessitates search for new therapeutic compounds for better management of epileptic disorders [20].

Since there are some reports on the use of constituents of B integerrima L. for the treatment of central nervous system diseases and its sedative effect, the anticonvulsant activity of methanolic extract, hydromethanolic fraction, and chloroform fraction of B integerrima was evaluated in this study.

2. Materials and methods

2.1. Animals

The study was performed on male albino mice, weighing 25 ± 1 g. Animals were housed in a ventilated room under a 12/12-hour light/dark cycle at 24 ± 2°C and had free access to water and food. All animal experiments were carried out in accordance with Mashhad University of Medical Sciences, Ethical Committee Acts.

2.2. Plant

The root of B integerrima was collected from Chenaran (Khorasan Province), Iran, and was identified by Mr Joharchi; voucher samples was preserved for reference in the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Mashhad (Voucher no. 36-0209-1).

2.3. Preparation of extract

B integerrima root was cleaned, dried in shadow, and powdered by a mechanical grinder. For the methanolic extract, the powder (35 g) was macerated in 1000 mL methanol for 7 hours, and the mixture was subsequently filtered and concentrated in vacuum at 40°C. The residue was suspended in normal saline. The concentrated extract was then fractionated with an equal volume of hydromethanolic or chloroform extract three times, to produce two fractions containing nonpolar and polar compounds, respectively.

2.4. Acute toxicity

Different doses of methanolic extracts were injected intraperitoneally into groups of six mice. The number of deaths that occurred after 48 hours of administration was

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**Table 1** Effect of methanolic extract of *B. integerrima* on PTZ-induced seizure in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Onset time of HLTE (s)</th>
<th>Mortality protection after 30 min (convulsion survivors/animals tested)</th>
<th>Mortality protection after 24 h (convulsion survivors/animals tested)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline (10 mL/kg)</td>
<td>163 ± 5.7</td>
<td>0/8</td>
<td>0/8</td>
</tr>
<tr>
<td>Diazepam (1 mg/kg)</td>
<td>1234 ± 27.7***</td>
<td>6/8**</td>
<td>6/8**</td>
</tr>
<tr>
<td>Methanolic extract (20 mg/kg)</td>
<td>251.1 ± 14.4</td>
<td>0/8</td>
<td>0/8</td>
</tr>
<tr>
<td>Methanolic extract (80 mg/kg)</td>
<td>296.4 ± 23.9</td>
<td>0/8</td>
<td>0/8</td>
</tr>
<tr>
<td>Methanolic extract (140 mg/kg)</td>
<td>621.1 ± 31.3**</td>
<td>2/8</td>
<td>1/8</td>
</tr>
<tr>
<td>Methanolic extract (200 mg/kg)</td>
<td>612.7 ± 19.3**</td>
<td>2/8</td>
<td>2/8</td>
</tr>
</tbody>
</table>

The methanolic extract and diazepam were administered 30 minutes prior to the injection of PTZ. Values are the mean ± SEM for eight mice.

**p < 0.01 and ***p < 0.001, as compared to control (normal saline), Tukey–Kramer. HLTE = hind limb tonic extension; PTZ = pentylentetrazole; SEM = standard error of mean.
considered. LD50 values and corresponding confidence limits were determined by the Litchfield and Wilcoxon methods (PHARM/PCS Version 4).

2.5. Anticonvulsant activity

2.5.1. Pentylenetetrazole-induced seizure test

The mice were randomly divided into 10 groups of eight animals each: (1) negative control group with normal saline (10 mL/kg), (2) positive control group with diazepam (1 mg/kg), (3, 4, 5, 6) methanolic extract-treated groups (20, 80, 140, and 200 mg/kg, respectively), (7, 8) hydromethanolic fraction-treated groups (140 and 200 mg/kg, respectively), and (9) chloroform fraction-treated group (140 and 200 mg/kg, respectively). The mice were given methanolic extract, hydromethanolic fraction, chloroform fraction, and controls, intraperitoneally, 30 minutes prior to the administration of 90 mg/kg pentylenetetrazole (PTZ). The animals were placed individually in plastic boxes and observed immediately after PTZ injection for a period of 30 minutes and after 24 hours. The onset time of hind limb tonic extensions (HLTEs) and the ratio of convulsion survivors to total animals tested (mortality protection) were recorded [21,22].

2.5.2. Maximal electroshock seizure test

The mice were randomly divided into 10 groups of eight animals each: (1) negative control group with normal saline (10 mL/kg), (2) positive control group with diazepam (1 mg/kg), (3, 4, 5, 6) methanolic extract-treated groups (20, 80, 140, and 200 mg/kg, respectively), (7, 8) hydromethanolic fraction-treated groups (140 and 200 mg/kg, respectively), and (9, 10) chloroform fraction-treated groups (140 and 200 mg/kg, respectively). The mice were given methanolic extract, hydromethanolic fraction, chloroform fraction, and controls, intraperitoneally, 30 minutes prior to the induction of maximal electroshock (MES). Then, the stimulus train was applied via ear-clip electrodes (sinusoidal pulses, 120 mA and 60 Hz, for 0.2 seconds) using a constant current stimulator (Digital Electroshock Model 150, EghbalTeb Co., Mashhad, Iran). A drop of 0.9% saline solution was applied on each ear of the animal prior to placing the electrode. The duration of HLTE, and the protection against the incidence of seizure, and the rate of convulsion survivors to total animals tested (mortality protection) were recorded [21,22].

2.6. Phytochemical screening

Phytochemical screening of the methanolic extract of B. integerrima was performed using the following reagents and chemicals: alkaloids with Dragendorff’s reagent, flavonoids with the use of Mg and HCl, tannins with 1% gelatin and 10% NaCl solutions, and saponins that can produce suds and induce hemolysis reaction [23].

2.7. Statistical analysis

The data were expressed as mean values ± standard errors of mean (SEMs) and tested with analysis of variance followed by the multiple comparison test of Tukey–Kramer.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Effect of hydromethanolic fraction of B integerrima on PTZ-induced seizure in mice.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Onset time of HLTE (s)</td>
</tr>
<tr>
<td>Normal saline (10 mL/kg)</td>
<td>225.1 ± 86.8</td>
</tr>
<tr>
<td>Diazepam (1 mg/kg)</td>
<td>1248.3 ± 178***</td>
</tr>
<tr>
<td>hydromethanolic fraction (140 mg/kg)</td>
<td>416 ± 78.5</td>
</tr>
<tr>
<td>hydromethanolic fraction (200 mg/kg)</td>
<td>542.3 ± 80.8**</td>
</tr>
</tbody>
</table>

The hydromethanolic fraction and diazepam were administered 30 minutes prior to the injection of PTZ. Values are the mean ± SEM for eight mice.

*p < 0.05, ** p < 0.01, and ***p < 0.001, as compared to control (normal saline), Tukey–Kramer. Fisher test for mortality protection. HLTE = hind limb tonic extension; PTZ = pentylenetetrazole; SEM = standard error of mean.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Effect of chloroform fraction of B integerrima on PTZ-induced seizure in mice.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Onset time of HLTE (s)</td>
</tr>
<tr>
<td>Normal saline (10 mL/kg)</td>
<td>202.8 ± 15.8</td>
</tr>
<tr>
<td>Diazepam (1 mg/kg)</td>
<td>1184.3 ± 69.7***</td>
</tr>
<tr>
<td>Chloroform fractions (140 mg/kg)</td>
<td>363.2 ± 19.3</td>
</tr>
<tr>
<td>Chloroform fractions (200 mg/kg)</td>
<td>415.3 ± 21*</td>
</tr>
</tbody>
</table>

The chloroform fraction and diazepam were administered 30 minutes prior to the injection of PTZ. Values are the mean ± SEM for eight mice.

*p < 0.05, ** p < 0.01, and ***p < 0.001, as compared to control (normal saline), Tukey–Kramer. Fisher test for mortality protection. HLTE = hind limb tonic extension; PTZ = pentylenetetrazole; SEM = standard error of mean.
Anticonvulsant effect of *Berberis integerrima*

### Table 4: Effect of methanolic extract of *B. integerrima* on MES-induced seizure in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Duration of HLTE (s)</th>
<th>Protection against seizure</th>
<th>Mortality protection after 30 min (convulsion survivors/animals tested)</th>
<th>Mortality protection after 24 h (convulsion survivors/animals tested)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline (10 mL/kg)</td>
<td>15.6 ± 0.2</td>
<td>0/8</td>
<td>6/8</td>
<td>6/8</td>
</tr>
<tr>
<td>Diazepam (1 mg/kg)</td>
<td>10.6 ± 0.25***</td>
<td>4/8*</td>
<td>8/8</td>
<td>8/8</td>
</tr>
<tr>
<td>Methanolic extract (20 mg/kg)</td>
<td>15.3 ± 0.18</td>
<td>0/8</td>
<td>6/8</td>
<td>6/8</td>
</tr>
<tr>
<td>Methanolic extract (80 mg/kg)</td>
<td>14.8 ± 0.27</td>
<td>0/8</td>
<td>7/8</td>
<td>7/8</td>
</tr>
<tr>
<td>Methanolic extract (140 mg/kg)</td>
<td>13.3 ± 0.18</td>
<td>1/8</td>
<td>7/8</td>
<td>7/8</td>
</tr>
<tr>
<td>Methanolic extract (200 mg/kg)</td>
<td>12.1 ± 0.28</td>
<td>2/8*</td>
<td>8/8</td>
<td>7/8</td>
</tr>
</tbody>
</table>

The methanolic extract and diazepam were administered 30 minutes prior to induction of MES seizures. Values are the mean ± SEM for eight mice.

***p < 0.001 and *p < 0.05, as compared to control (normal saline), Tukey–Kramer. Fisher test for mortality protection. HLTE = hind limb tonic extension; MES = maximal electroshock; SEM = standard error of mean.

### Table 5: Effect of hydromethanolic fraction of *B. integerrima* on MES-induced seizure in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Duration of HLTE (s)</th>
<th>Protection against seizure</th>
<th>Mortality protection after 30 min (convulsion survivors/animals tested)</th>
<th>Mortality protection after 24 h (convulsion survivors/animals tested)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline (10 mL/kg)</td>
<td>16.25 ± 0.67</td>
<td>0/8</td>
<td>5/8</td>
<td>5/8</td>
</tr>
<tr>
<td>Diazepam (1 mg/kg)</td>
<td>10.6 ± 0.7**</td>
<td>4/8*</td>
<td>8/8*</td>
<td>8/8*</td>
</tr>
<tr>
<td>Hydromethanolic fraction (140 mg/kg)</td>
<td>13.6 ± 0.7</td>
<td>1/8</td>
<td>5/8</td>
<td>4/8</td>
</tr>
<tr>
<td>Hydromethanolic fraction (200 mg/kg)</td>
<td>13.3 ± 0.95</td>
<td>1/8</td>
<td>5/8</td>
<td>5/8</td>
</tr>
</tbody>
</table>

The hydromethanolic fraction and diazepam were administered 30 minutes prior to induction of MES seizures. Values are the mean ± SEM for eight mice.

* *p < 0.05 and ** *p < 0.01, as compared to control (normal saline), Tukey–Kramer. Fisher test for mortality protection. HLTE = hind limb tonic extension; MES = maximal electroshock; SEM = standard error of mean.

Statistical significance was considered at $p < 0.05$ level. The percentage of mortality was assessed by Fisher’s exact test.

### 3. Results

#### 3.1. Acute toxicity

At a dose of 200 mg/kg, the methanolic extract decreased locomotion in mice for almost 15 minutes and then the mice were returned to normal condition. The LD₅₀ value of the methanolic extract was 302.676 mg/kg (95% CL: 243–359) and the maximum nonfatal dose was 200 mg/kg.

#### 3.2. Anticonvulsant activity

##### 3.2.1. PTZ-induced seizure test

In the PTZ-induced seizure, administration of the methanolic extract at doses of 140 and 200 mg/kg ($p < 0.01$), hydromethanolic fraction at a dose of 200 mg/kg ($p < 0.01$), and chloroform fraction at a dose of 200 mg/kg ($p < 0.01$) increased the onset time of HLTEs compared to the negative control group. The rate of convulsion survivors to total animals tested (mortality protection) was 2/8 in methanolic extract, and 3/8 in hydromethanolic fraction at a dose of 200 mg/kg and in chloroform fraction at a dose of 140 mg/kg, which was less than the efficacy of diazepam. In addition, the effect of methanolic extract was more than other fractions (Tables 1–3).

##### 3.2.2. MES test

In the MES test, the methanolic extract, and hydromethanolic and chloroform fractions reduced the duration of HLLE, but this effect was not significant. According to the data, the methanolic extract and both fractions exhibited protective effects against mortality in MES experiments (Tables 4–6).

##### 3.2.3. Phytochemical screening

Phytochemical screening of the methanolic extract indicated the presence of alkaloids and tannins.

### 4. Discussion

In respect to LD₅₀ values, the methanolic extract of *B. integerrima* root was compared with a toxicity classification [24], this extract is considered very toxic. The results of this study demonstrated that the methanolic extract and hydromethanolic and chloroform fractions of *B integerrima* have anticonvulsant activity. Data showed that the methanolic extract and both hydromethanolic and chloroform fractions displayed anticonvulsant effect in the PTZ-induced seizure model. In fact, compounds with anticonvulsant properties in the petit mal epilepsy were effective in PTZ-induced seizure experiment [25].
Therefore, *B. integerrima* root could be beneficial in petit mal epilepsy. In the MES test, the methanolic extract and both fractions of *B. integerrima* did not show any significant effects in reducing HLTE duration. Thus, *B. integerrima* has no protective activity against the grand mal epilepsy. As this plant exhibited more antiepileptic activity in the PTZ-induced seizure test than in MES test, it could be more useful against petit mal epilepsy.

The anticonvulsant activity of *B. integerrima* may be attributed to the presence of alkaloids and tannins, which have been found in its methanolic extract by phytochemical investigation. The methanolic extract was more effective than chloroform fraction in petit mal epilepsy, suggesting that more effective constituent(s) may be more polar. Previous studies have demonstrated that some alkaloids have anticonvulsant activity [26,27]. Berberine is an isoquinoline alkaloid, present in root and stem bark of *Berberis* species [4,7], and may be responsible for this useful effect. It was shown that berberine has various beneficial effects on the activities of central nervous system, such as protective effect in mental depression, anxiety, and sedative effect [16,18]; the anticonvulsant effect of this plant may be related to the central effect of its constituents. Recently, Bhutada et al. [28] demonstrated the anticonvulsant effect of berberine in mice. Consistent with this study, our results showed that methanolic extract, in addition to hydromethanolic and chloroform fractions of *B. integerrima*, had anticonvulsant activity. As the extracts of *B. integerrima* contain many constituents, this effect could be related to some other constituents in addition to berberine. Thus, further studies are needed to identify other constituents that may have effective anticonvulsant activities.

In addition, the antiepileptic effect of several compounds is related to neuronal calcium channel inhibition; other studies demonstrated that berberine inhibited norepinephrine-, K+-, and H2O2-induced [Ca2+] elevation [29]. Therefore, the protective effect of *B. integerrima* might be due to its inhibitory activity on calcium channel as well.

The present study demonstrated that methanolic extract (more effective), and hydromethanolic and chloroform fractions of *B. integerrima* have anticonvulsant activity in PTZ-induced seizures in mice. Therefore, *B. integerrima* may be more useful in petit mal epilepsy. This may lead to the utilization of this plant as a therapeutical agent for treating epilepsy or/and as a supplement to more conventional anticonvulsant drugs.

**Acknowledgments**

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**References**


